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CYANONEWS

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CYANONEWS - a newsletter intended to provide cyanobacteriologists with a forum for rapid informal communication, unavailable through journals. Everything you read in this newsletter is contributed by readers like yourself. Published occasionally, about three times per year.

SUBSCRIPTIONS - $8/year. (See last page)

CONTRIBUTIONS - Expected every couple of years: a new result, an upcoming meeting or a summary of a past meeting, a post-doctoral opening, a new publication, a request for strains, a change of life... something. See last page for addresses you can send news to.

HOW TO FIND OUT MORE ABOUT SOMETHING YOU READ HERE - Contact the person whose name is capitalized in the news item. Addresses are given at the end of the issue. Also, a Directory of Cyanobacteriologists is distributed every two years. If you need one, write to Jeff Elhai (see last page of newsletter).

INSTRUCTIONS TO AUTHORS - Send news.

NEWS
* BTI toxin expressed in *Synechocystis*
* Chlorophyll metabolism, gabaculin action
* New genes of chromatic adaptation
* Response of *Anacystis* to nutrient deprivation
* Organization of RNA polymerase genes
* Highly fluorescent unicellular cyanobacteria
* Mysterious open reading frames found in excision element of *Anabaena*
* Genome of *A. quadruplicatum* mapped
* Synthesis of Adda, amino acid peculiar to toxins
* N_2-fixing cyanobacterium associated with rice

ITEMS OF INTEREST
* Microfuge miniprep for *Synechocystis* PCC 6803 chromosomal DNA
* Directory of electronic mail addresses

BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BULLETIN BOARD*BOARD

A new DIRECTORY OF CYANOBACTERIOLOGISTS will be prepared and distributed early next year. If you are not in it, or your entry has changed or was in error, now is the time to say so. Contact:

Jeff Elhai, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A. (Tel) 517-353-6641. (EMail)Cyano@MSU.Bitnet. (FAX) 517-353-9168.

A NATO Advanced Research Workshop on "THE MARINE DIAZOTROPH *TRICHODESMIUM*: BIOLOGY AND ECOLOGY" will be held in Bamberg, Germany, May 25-30, 1991. Those interested in attending or presenting a paper should contact:

E.J. Carpenter, Marine Sciences Research Center, State University of New York, Stony Brook, New York, 11794-5000, U.S.A. (Tel) 516-632-8696. (EMail) ecarpenter@SBCCMAIL.Bitnet.

The VII INTERNATIONAL SYMPOSIUM ON PHOTOSYNTHETIC PROKARYOTES will take place in Amherst, Massachusetts U.S.A. July 21-26, 1991. The program has been divided into five themes: (1) photosynthetic apparatus, (2) reaction center/antenna complexes, (3) metabolic systems, (4) ecology and systematics, and (5) biochemical processes. Conference expenses will range from $433.50 U.S. (less for students) to $633.50, depending on room choice. The organizers are trying very hard to find money to pay expenses for at least some applicants without access to hard currency. Contact:

Clint Fuller, Department of Biochemistry, University of Massachusetts, Amherst, MA U.S.A. (Tel) 413-545-0328, (FAX) 413-545-3291, (EMail) Biblio@hampvms.

Olav Skulberg has put together a list of TOXIGENIC SPECIES of cyanobacteria, consisting of 39 strains and references for each. Any scientists interested in this list or in material describing the culture collection of the Norwegian Institute for Water Research (NIVA) can contact him at:

Olav Skulberg, Norwegian Institute for Water Research, P.O. Box 69 Korsvoll, N-0808 Oslo 8, NORWAY. (Tel) 472-235280. (FAX) 472-394189.

Rosi Rippka and Mike Herdman have almost completed a CATALOGUE OF STRAINS HELD IN THE PASTEUR CULTURE COLLECTION. The catalogue lists more than 400 axenic strains available from the PCC and will be available as of January 1991 for a small fee (yet to be decided) to cover part of the costs of production and mailing. Strains are listed in numerical order by PCC number and in alphabetical order by genus. The catalogue gives for each strain its history, isolator, habitat, strain number in other culture collections, and medium used for cultivation. It provides, as well, useful information of a more general nature: how to order strains from the PCC, how to deposit cultures, a description of the taxonomy employed, a list of reference strains and those appropriate for teaching, a quick reference table to other culture collections (including the state of purity of the strains therein), a list of synonyms and, finally, useful references. Contact:

Rosi Rippka and Mike Herdman, Physiologie Microbienne, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15. (Email) Cyano@Pasteur.Bitnet

Bill Widger is looking for GENES TO USE AS PROBES in converting the physical map of *Agmenellum quadruplicatum* (*Synechocystis*) PCC7002 into a genetic map (See NEWS). Presently, he has probes for most genes encoding components of PSI, PSII, and the cytochrome b_6/f complex, as well as those related to phycocyanin and allophycocyanin, but he has very few genes not related to photosynthesis. If you would be willing to supply probes he's sure appropriate arrangements can be made. Contact:

Bill Widger, University of Houston, Dept. of Biology and Biophysical Sciences, SR #1, 4800 Calhoun Rd., Houston TX 77004. (Tel) 713-749-4396, (EMail-Bitnet) WIDGER@UHOU, (EMail-Internet) WIDGER@UH.EDU.

"ALGAL AND CYANOBACTERIA BIOTECHNOLOGY" is a recently published book that focuses on commercial possibilities of algae and cyanobacteria and their products. It was edited by R.C. Cresswell, T.A.V. Rees, and N. Shah and costs $120 (U.S.) for 341 pages. Contact:

Longman Scientific and Technical, Harlow, Essex, U.K.
or John Wiley & Sons, Inc., New York, U.S.A.

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LAMONT ANDERSON has left his post-doctoral position in Arthur Grossman's laboratory at Stanford University and found a real job, as a faculty member at University of Tulsa. He's got a new job, a new lab, a new grant, and a new FAX board attached to his computer (which you are invited to use). He also has a lot of new teaching resonsibilities, which works against new results very soon.

Department of Biological Sciences, University of Tulsa, 600 S.College Ave., Tulsa, OK 74104-3189, U.S.A. (Tel) 918-631-3328. (FAX) 918-631-3328.

BERGITTA BERGMAN has left University of Uppsala and taken a position as professor and head of plant physiology at Stockholm University.

Department of Botany, Stockholm University, S-106 91 Stockholm, SWEDEN, (Tel) 46-8-16 37 51, (FAX) 46-8-16 55 25].

JOHN ERIKSSON has shifted operations from Abo Akademi to Northwestern University. He'll continue his work on cyanobacterial toxins.

Dept. of Cell, Molecular, and Structural Biology, Northwestern University Medical School, 303 E. Chicago Ave, Chicago, IL 60611-3008,
(Tel) 312-503-4277, (FAX) 312-908-0954.

ANNELIESE ERNST has begun a two year stay in the laboratory of Peter Wolk at Michigan State University, where she will extend her work done at University of Konstanz on oxygen protection and metabolism within heterocysts of *Anabaena* to the realm of genetics.

MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 U.S.A.
(Tel) 517-353-6641, (FAX) 517-353-9168, (EMail) 22333ALE @ MSU.Bitnet

FRITZ JÜTTNER has moved from the Max Planck Institut für Limnologie to the Limnological Station of University of Zürich.

Universität Zürich, Limnologische Station, Seestr.187, CH-8802 Kilchberg, SWITZERLAND.
(Tel) 01-715-2905. (FAX) 01-715-5165.

BOB RAMAGE has left University of Arizona for the University of Illinois. He still intends to work on Rubisco.

Department of Agronomy, S-215 Turner Hall, 1102 S.Goodwin Ave., Urbana, IL 61801 U.S.A.
(Tel) 217-244-3080.

STEVE ROBINSON has announced his intention to leave the relative safety of academic life at the University of Massachussetts to let the winds buffet him where they may. His decision will take effect at the end of the International Symposium on Photosynthetic Prokaryotes next summer. He doesn't know what he'll do next. Maybe write a textbook. Maybe get a research position. But he knows what he won't be doing: running from committee to committee and around in the academic circle he's learned he can do without.

Department of Botany, University of Massachusetts, Amherst, MA 01003, U.S.A. (Tel) 413-545-4380.

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BTI TOXIN EXPRESSED IN *SYNECHOCYSTIS*

WIPA CHUNGJATUPORNCHAI tells us of her progress in expressing an insecticidal-protein in cyanobacteria, with the ultimate aim of using the resulting organism to control natural mosquito populations. A gene (*bt8*) taken from *Bacillus thuringiensis* subsp. israeliensis (Bti), encoding a Bti toxin, was placed under transcriptional control of P_{psbA}, derived from the chloroplast of tobacco. This gene fusion was directed to a specific site in the chromosome of *Synechocystis* PCC6803. Total protein derived from the recombinant organism (but not from the wild-type strain) proved toxic to mosquito larva, but the organism itself was not mosquitocidal. Bti toxin comprised about 0.1% of the total protein. P_{psbA} was fused also to *bar*, a gene determining resistence to the herbicide phosphinothricin. P_{psbA}-*bar* produced two-fold higher steady state levels of mRNA than P_{psbA}-*bt8*, sufficient to confer herbicide resistence on the cyanobacterium. The work points out problems of expression that must be overcome before cyanobacterially-borne Bti toxin can be employed as a mosquitocidal agent. A report of this work will appear in Current Microbiol.

CHLOROPHYLL METABOLISM, GABACULIN ACTION DISSECTED

ARNOLD SMITH has summarized recent work that he, Lyndon Rogers, and Alan Bull have done (in collaboration with Gamini Kannangara and his colleagues in the department of Physiology of the Carlsberg Laboratory, Copenhagen) on the early stages of chlorophyll biosynthesis. Glutamate semialdehyde aminotransferase catalyzes the final step in the synthesis of 5-aminolevulinic acid, a precursor of chlorophyll and other porphoryins. The enzyme was isolated from *Synechococcus* PCC6301 as a single subunit enzyme of 46 kdal dependent on pyridoxamine phosphate for activity. The gene encoding this enzyme was cloned and sequenced. A large open reading frame was identified that begins with the unusual initiation codon TTG. The predicted amino acid sequence of this open reading frame is 72% identical to that of the enzyme from barley and contains a possible binding site for the B6 cofactor. In order to understand the nature of resistence to gabaculine, an inhibitor of chlorophyll porphyrin, the gene encoding the aminotransferase from the gabaculine-resistant strain GR6 was cloned and sequenced. The most notable differences are a deletion near the 5' end of the gene, spanning nine nucleotides, and a single base transition in a conserved sequence near the region encoding the putative B6-binding site. Further studies are being directed to identifying the effect of these and other changes on the sensitivity of the gene product to gabaculine.

COSMID LIBRARY IDENTIFIES NEW GENES OF CHROMATIC ADAPTATION

JOHN COBLEY sends compliments from his laboratory, which has constructed a cosmid library for the purpose of studying chromatic adaptation in *Fremyella* diplosiphon (also known as *Calothrix* PCC7601). The library is based on a new 11.9 kb vector, pJCF8101, which can replicate in both *E.coli* and *F.diplosiphon.* Each of of 2000 individual members contain inserts of about 35 kb taken at random from the DNA of *F.diplosiphon.* The clones are maintained separately in a strain of E.coli carrying the helper plasmid, pJCF17, permitting conjugation into *F.diplosiphon.*

Some interesting cosmids were identified by their ability to alter the properties of wild-type *F.diplosiphon* when introduced into the strain. Some cosmids caused the synthesis of phycoerythrin to decrease in green light, a property shared by plasmids carrying genes for phycocyanin or allophycocyanin. Another cosmid (called pIX-G10) caused an increase in phycoerythrin synthesis. Since subclones from the cosmid that have a similar effect do not carry the genes encoding phycoerythrin or related linker polypeptides, John suspects that some gene on pIX-G10 may affect the normal transduction of a green light signal. Finally, two overlapping cosmids have been identified that complement the mutant *F.diplosiphon* SF48, in which phycoerythrin fails to attach to phycobilisomes. Neither of the cosmids carry genes encoding phycoerythrin-related linkers, and a fragment encoding the phycoerythrin genes fails to complement the mutant, leading to the conclusion that the defect of SF48 is in a new gene involved in the assembly of phycobilisomes in green light.

RESPONSE OF *ANACYSTIS* TO NUTRIENT DEPRIVATION

JACKIE COLLIER has been looking at the bleaching response of *Anacystis nidulans* R2 to macronutrient deprivation and tells us what she has seen. The responses to deprivation for nitrogen or sulfur are similar in that there's a decrease in mRNA abundance for phycobilisome components and an ordered breakdown of phycobilisomes that are already present. The cellular content of chlorophyll decreases by dilution as the cells divide. The ordered breakdown of phycobilisomes doesn't seem to occur in the case of phosphate deprivation. She has also isolated a few nonbleaching mutants, which have very interesting phenotypes. Although all were selected for their abilities to stay green on sulfate-free plates, preliminary results indicate that they've got various combinations of bleaching defects in media lacking nitrogen, sulfur, or phosphate. She hopes to complement these mutants to get some insight into the control over the bleaching process.

NOVEL ORGANIZATION OF CYANOBACTERIAL RNA POLYMERASE GENES

MALCOLM POTTS passes on an interesting result from the Ph.D. thesis of Wen-Qin Xie, concerning the *rpo* genes (encoding RNA polymerase) of *Nostoc commune.* In *E.coli* and other eubacteria, archaebacteria, and plant chloroplasts, the genes encoding RNA polymerase are cotranscribed. Wen-Qin found that this is not the case in *N.commune.* In this cyanobacterium, *rpoB, rpoC1,* and *rpoC2* are clustered on the chromosome, but *rpoB* is transcribed separately from *rpoC1C2.* Work related to the *rpoC1C2* promoter will appear in Arch. Biochem. Biophys. (Xie and Potts). Recent work suggests that water stress regulates expression of the *rpo* genes at the level of transcription.

HIGHLY FLUORESCENT UNICELLULAR CYANOBACTERIA FROM LAKE CONSTANCE

ANNELIESE ERNST Ernst reports the isolation of several unicellular cyanobacteria in the laboratory of Peter Böger, Konstanz, Germany. The isolates originate from the picoplankton fraction of Lake Constance (a 550 km^2 large prealpine lake in central Europe). The picoplankton of this lake is dominated by highly fluorescent phycoerythrin (PE)-rich cyanobacteria [T. Weisse, J Plankton Res (1988) 10:1179-1188]. All PE-rich isolates exhibit an in vivo fluorescence absorbance spectrum similar to that of a strain of *Synechococcus rubescens* originating from Lake Zürich [T.-P. Chang, Schweiz Z Hydrol (1980) 42:247-254], to that of marine *Synechococcus* WH 7805, and to other strains without phycourobilin of the marine cluster A as defined in *Bergey's Manual of Systematic Bacteriology* (Vol. 3, 1989) . Some of the PE-rich isolates appear identical to *Synechococcus rubescens* and two are morphologically similar to *Synechococcus elongatus* PCC6716, a thermophilic cyanobacteria with phycocyanin (PC)-rich phycobilisomes.

Another highly fluorescent species was found to contain PC and possibly traces of a PE-like pigment but no allophycocyanin. This strain was provisionally assigned to *Synechocystis*, although it differs in several respects from all strains of this genus described thus far: (1) Morphology: electron micrographs, performed by Patricia Gimenez on her stay in Konstanz and by W. Reuter in Werner Wehrmeyer's laboratory (Marburg, Germany) indicate closely stacked thylakoids and a peculiar cell wall; (2) Pigmentation: the carotenoid composition [Gerhard Sandmann, Konstanz] is quite unusual, and light acquisition by chlorophylls is more effective [A. Ernst]. Last but not least, the strain fixes molecular nitrogen under anaerobic conditions [Susanne Brass, Konstanz].

MYSTERIOUS OPEN READING FRAMES FOUND IN EXCISION ELEMENT OF ANABAENA

PETER LAMMERS describes progress in understanding the role of the 11kb element that interrupts the nifD gene (encoding a subunit of nitrogenase) in *Anabaena* PCC7120. This element is excised in heterocysts during the course of heterocyst differentiation. Excision is essential for expression of nitrogenase, but it is not clear why the element should be there in the first place. In sequencing this region, Peter and coworkers identified four open reading frames in the same orientation as *xisA*, a gene previously shown to be required for excision. One of the four open reading frames is capable of encoding a protein very similar to cytochrome P-450 omega-hydroxylase, especially in functionally significant domains. A paper describing this work will appear in the December 1990 issue of Journal of Bacteriology.

GENOME OF *AGMENELLUM QUADRUPLICATUM* MAPPED

BILL WIDGER announces that a genomic restriction map of *Agmenellum quadruplicatum* (*Synechocystis*) PCC7002 is about 95% complete. Digestion by NotI produces 21 fragments ranging in size from 440,000 bp to 4,000 bp while SalI produces 34 fragments ranging from 280,000bp to 2,000bp. Summation of the fragment sizes gives a genome size of 2.7 Mbp. The NotI and SalI sites were ordered by identifying cloned DNA fragments that span the restriction sites or by cross hybridizing the NotI and SalI fragments. A lambda library was constructed from *Agmenellum* DNA partially digested with Sau3AI, and about 1600 clones have been mapped to one or more of the large restriction fragments. Ordering of the clones within the restriction fragments is in progress. In parallel, he is attempting to convert the physical map to a genetic map, using cloned genes as probes (see BULLETIN BOARD).

ADDA, AMINO ACID PECULIAR TO TOXINS, SYNTHESIZED

KENNETH RINEHART summarizes the present state of his work on the isolation and characterization of bioactive compounds from marine plants and animals, done in collaboration with Wayne Carmichael and Val Beasley. Work has recently focused on nodularin, a cyclic pentapeptide from *Nodularia spumigena*, and microcystin-LR, a cyclic heptapeptide from *Microcystis aeruginosa*. Both compounds are hepatotoxins. In the case of nodularin, they identified the amino acids and sequenced them. In the case of microcystin-LR, they confirmed the amino acid content and sequence. In both cases, fast atom bombardment and tandem fast atom bombardment mass spectrometry played major roles in the assignments. Adda, a unique twenty-carbon amino acid, was found in both toxins as well as other microcystins. K.R.'s group has succeeded in synthesizing Adda, partly to confirm their earlier work on the stereochemistry of the compound, but mainly to test its biological activity and to initiate synthesis of the complete toxins.

ASSOCIATION OF RICE WITH NITROGEN-FIXING CYANOBACTERIUM REPORTED

NATALIA KOZYROVSKAYA sends provocative news about a nitrogen-fixing cyanobacterial strain, *Anabaena thermalis*, that was isolated from surface-sterilized leaves and stems of wild floating rice in Viet Nam. She and coworkers inoculated sterilized rice seeds with cultures of *A. thermalis* and found the cyanobacterium was able to invade root hairs, root parenchyma, intercellular spaces, and xylum of the rice plant. Inoculation of rice with the cyanobacterium affected growth of the plant in that biomass was higher but the weight of rice grains was less than the control.

ELECTRONIC MAIL ADDRESS DIRECTORY

* All addresses have the .Bitnet extension unless otherwise noted.
* Many addresses are shared, so it is advisable to show the intended addressees name as the Subject
* Spaces have been placed around "@" to improve readability, but omit them when using the address.
* Please send all additions and corrections to: Cyano@Msu.Bitnet

Cyanobacteriologist	Institution	Electronic Mail Address
Yael Avissar	Brown U., Providence USA	me599004 @ Brownvm
Chris Bauer	U.Chicago, USA	ccb1 @ Midway.UChicago.Edu
Sam Beale	Brown U., Providence USA	me599017 @ Brownvm
John Biggins	Brown U., Providence USA	me59901 @ Brownvm
Todd Black	Michigan St.U., USA	22333tab @ MSU
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Cathy Cook	U.Thessaloniki, Greece	cdaz0204 @ Grtheun1
Raymond Cox	Odense U., Denmark	jmo @ Dou.dk
Bill Cramer	Purdue U., USA	membrane @ Purccvm
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Jim Golden	Texas A&M, USA	jwg2076 @ TamVenus
John Golbeck	Portland St. U., USA	bchm005 @ Unlvm
Susan Golden	Texas A&M, USA	ssg7231 @ TamVenus
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Aaron Kaplan	Hebrew U., Jerusalem Israel	AaronKa @ Hujivms
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Martin Mulligan	Memorial U.,Newfoundland Canada	Mulligan @ Kean.Mun.CA
Norio Murata	Natl.Inst.Basic Biol.,Okazaki Japan	Murata @ Jpnonri
Kaori Ohki	Natl.Inst.Basic Biol.,Okazaki Japan	Ohki @ Jpnonri
Himadri Pakrasi	Washington U., St. Louis USA	Pakrasi_H @ Wums
Jean-Michel Panoff	Michigan State U, USA	22333jmp @ Msu
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Judy Philbrick	Rutgers U., New Jersey USA	Philbrick @ Biovax
Malcolm Potts	Virginia Polytech.Inst., USA	PottsM @ Vtvm1
T.S.Ramasubramanian	Texas A&M, USA	tsr5081 @ TamVenus
Rosi Rippka	Inst.Pasteur, Paris France	Cyano @ Pasteur
Michael Schaefer	Stanford U., USA	w5.c38 @ Stanford
Georg Schmetterer	U.Wien, Vienna Austria	a8422dad @ Awiuni11
Walter Sidler	Inst.Molec.Biol., Zürich Switzerland	Molekula @ Czheth5a
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Geoffrey Smith	Austr.Natl.U., Canberra Australia	gds658 @ Csc.anu.oz.au
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Eduardo Fernandez Valiente	U.Autonoma Madrid, Spain	Quesada @ Emduam11
Wim Vermaas	U.Arizona, USA	atwfv @ Asuacad
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Peter Wolk	Michigan St.U., USA	22333cpw @ Msu
Michelle Wood	U.Chicago, USA	OMNET:M.Wood

MICROFUGE MINIPREP FOR *SYNECHOCYSTIS* PCC6803 CHROMOSOMAL DNA

Earlier this year DEXTER CHISHOLM passed on a protocol that allows the isolation of moderate amounts of DNA from *Synechocystis* PCC6803. After the protocol was published in the newsletter, he received calls from people who had tried the protocol with little success. Further investigation revealed that these people had carefully followed the protocol exactly as printed in the newsletter, which, unfortunately, was NOT exactly as written by D.C. Therefore, far from casting doubt on the efficacy of this protocol, the failure of these workers to get any significant quantitites of DNA emphasizes the peril of modifying even a single one of its steps. The true protocol is printed below.

SUMMARY: The procedure allows cells from either plates or liquid culture to be used and confines all manipulations to 1.5 ml microcentrifuge tubes. Cell lysis is achieved using lysozyme, sarkosyl, and phenol. Polysaccharides are removed by CTAB extraction. He and others have been using this procedure for many months with consistent results. The DNA restricts well and supports PCR amplification. The procedure also scales up well.

HARVEST CELLS: Spin down 12 ml of culture (OD_{730} of at least 2.0), or scrape a pea-sized glob of cells from a healthy plate. Resuspend in 400 ul of TES in a microfuge tube.

DIGEST WITH LYSOZYME: Add 100 ul of lysozyme (@ 50 mg/ml) and incubate for 15 min at 37° (mix occasionally because cells settle out).

LYSE WITH SARKOSYL AND PHENOL: Add 50 ul 10% sarkosyl, and then add 600 ul phenol and torture on a rotating wheel for 15 min.

REMOVE DEBRIS: Spin in microfuge for at least 5 min. Transfer aqueous upper layer to new tube.

DIGEST WITH RNase: Add 5 ul of 2 U/ul RNase (Boehr.-Mannheim #1119-915). Incubate 15 min at 37°.

WASH WITH CTAB: Add 100 ul of 5M NaCl, 100 ul of CTAB-NaCl solution, and 600 ul of chloroform. Extract on wheel for 15 min. Spin for 2 min, and transfer aqueous upper layer to new microfuge tube.

PRECIPITATE DNA: Add 700 ul isopropanol. Spin 15 min to pellet DNA.

RINSE PELLET: Rinse pellet with 70% ethanol. Dry in Speedvac. Resuspend in 100ul of TE. Use 10-20 ul per lane for Southern.

TES solution
- 2.5 ml 1M Tris, pH8.5 (5 mM)
- 5 ml 5M Nacl (50 mM)
- 5 ml 500 mM EDTA (5 mM)
- Bring volume to 500 ml

CTAB-NaCl Solution:
- 4.1 g NaCl in 80 ml water (700 mM)
- 10 g CTAB (10%)
- Requires heat to get into solution
- Bring volume to 100 ml

EVOLUTION, SYSTEMATICS, and PROCHLOROPHYTES

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ECOLOGY and SYMBIOSIS

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